



10025222.060302 #7

Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS
AUREUS GENE AND ITS ENCODED PROTEIN
Appl. No.: 10/025,222 Filing Date: 12/19/01 Applicant: Pelletier, et al.
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SEQ ID NO: 1

>STAAU_R009 nucleotide sequence: 1800
TTGCGAATAGATCAATCGATCATTAAATGAAATAAAAGATAAAACCGACATTTTAGACTTGGTAAGTGA
ATATGTAAAATTAGAAAAGAGAGGACGCAATTATATAGGTTTGTGTCCTTTTCATGATGAAAAGACAC
CTTCATTTACAGTTTCTGAAGATAAAACAAATTTGTCAATTGTTTTGGTTGTAAAAAAGGTGGCAATGTT
TTTCAATTTACTCAAGAAATTAAAGACATATCATTGTTGAAGCGGTAAAGAATTAGGTGATAGAGT
TAATGTTGCTGTAGATATTGAGGCAACACAATCTAACTCAAATGTTCAAATTGCTTCTGATGATTTAC
AAATGATTGAAATGCATGAGTTAATACAAGAATTTTATTATTACGCTTTAACAAAGACAGTCGAAGGC
GAACAAGCATTAAACGTACTTACAAGAACGTGGTTTTACAGATGCGCTTATTAAAGAGCGAGGCATTGG
CTTTGCACCCGATAGCTCACATTTTTGTGTCATGATTTTCTTCAAAAAAAGGGTTACGATATTGAATTAG
CATATGAAGCCGGATTATTATCACGTAACGAAGAAAATTTCAAGTTATTACGATAGATTTGAAATCGT
ATTATGTTTCCTTTGAAAAATGCGCAAGGAAGAATTGTTGGATATTCAGGTCGAACATATACCGGTCA
AGAACCAAAATACTTAAATAGTCCTGAAACACCTATCTTTCAAAAAAGAAAGTTGTTATACAACCTTAG
ATAAAGCGCGTAAATCAATTAGAAAATTAGATGAAATCGTATTACTAGAAGGTTTTATGGATGTTATA
AAATCTGATACTGCTGGCTTGAAAAACGTTGTTGCAACAATGGGTACACAGTTGTCAGATGAACATAT
TACTTTTATACGAAAGTTAACATCAAATATAACATTAATGTTTGATGGGGATTTTGCGGGTAGTGAAG
CAACACTTAAAACAGGTCAAAATTTGTTACAGCAAGGGCTAAATGTATTTGTTATACAATTGCCATCA
GGCATGGATCCGGATGAATACATTGGTAAGTATGGCAACGATGCATTTACTGCTTTTGTAAAAAATGA
CAAAAAGTCATTTGCACATTATAAAGTGAGTATATTAAGATGAAATTGCACATAATGACCTTTTCAT
ATGAACGTTATTTGAAAGAACTAAGTCATGATATTTTCGCTTATGAAATCATCGATTTTGCAACAAAAG
GCTTTAAATGATGTTGCACCATTTTTCAATGTTAGTCCTGAGCAATTAGCTAACGAAATACAATTCAA
TCAAGCACCAGCCAATTATTATCCAGAAGATGAGTATGGCGGTTACATTGAACCTGAGCCAATTGGTA
TGGCACAATTTGACAATTTGAGCCGTCAAGAAAAAGCGGAGCGAGCATTTTTAAAACATTTAATGAGA
GATAAAGATACATTTTAAATTATTATGAAAGTGTTGATAAGGATAACTTCACAAATCAGCATTTTAA
ATATGTATTCTGAAGTCTTACATGATTTTTATGCGGAAAATGATCAATATAATATCAGTGATGCTGTGC
AGTATGTTAATTCAAATGAGTTGAGAGAAACACTAATTAGCTTAGAACAATATAATTTGAATGACGAA
CCATATGAAAATGAAATTGATGATTATGTCAATGTTATTAATGAAAAAGGACAAGAAACAATTGAGTC
ATTGAATCATAAATTAAGGGAAGCTACAAGGATTGGCGATGTAGAATTACAAAAATACTATTTACAGC
AAATTGTTGCTAAGAATAAAGAACGCATGTAG

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SEQ ID NO: 2

>STAAU_R009 amino acid sequence : 599

LRIDQSIINEIKDKTDILDLVSEYVKLEKRGRNYIGLCPFHDEKTPSFTVSEDKQICHCFGCKKGGNV
FQFTQEIKDISFVEAVKELGDRVNVAVDIEATQSNSNVQIASDDLQMIEMHELIQEFYYYALTTKTVEG
EQALTYLQERGFTDALIKERGIGFAPDSSHCHDFLQKKGYDIELAYEAGLLSRNEENFSYYDRFRNR
IMFPLKNAQGRIVGYSGRITYTGQEPKYLNSPETPIFQKRKLLYNLDKARKSIRKLDEIVLLEGFMDVI
KSDTAGLKNVATMGTQLSDEHITFIRKLTSNITLMFDGDFAGSEATLKTGQNLLQQGLNVFVIQLPS
GMDPDEYIGKYGNDAFTAFVKNDKKSFAHYKVSILKDEIAHNDSL YERYLKELSHDISLMKSSILQQK
ALNDVAPFFNVSPEQLANEIQFNQAPANYYPEDEYGGYIEPEPIGMAQFDNLSRQEKAERAFLKHLMR
DKDTFLNYYESVDKDNFTNQHFKYVFEVLHDFYAENDQYNISDAVQYVNSNELRETLLISLEQYNLNDE
PYENEIDDYVNVINEKGQETIESLNHKLREATRIGDVELQKYYLQQIVAKNKERM

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FIGURE 2

SEQ ID NO: 3

>96ORF078 nucleotide sequence

ATGAATATAATGCAATTCAAAAGCTTATTGAAATCGATGTATGAAGAGACAAAGCAAAGC
GACCCGATTGTAGCAAATGTATATATCGAGACTGGTTGGGCGGTCAATAGATTGTTGGAC
AATAACGAGTTATCGCCTTTCGATGATTACGACAGAGTTGAAAAGAAAATCATGAATGAA
ATCAACTGGAAGAAAACACACATTAAGGAGTGTTAA

SEQ ID NO: 4

>96ORF078 amino acid sequence 96_NT|10148-10363|

MNIMQFKSLLKSMYEETKQSDPIVANVYIETGWAVNRLLDNNELSPFDDYDRVEKKIMNE
INWKKTHIKEC

FIGURE 3

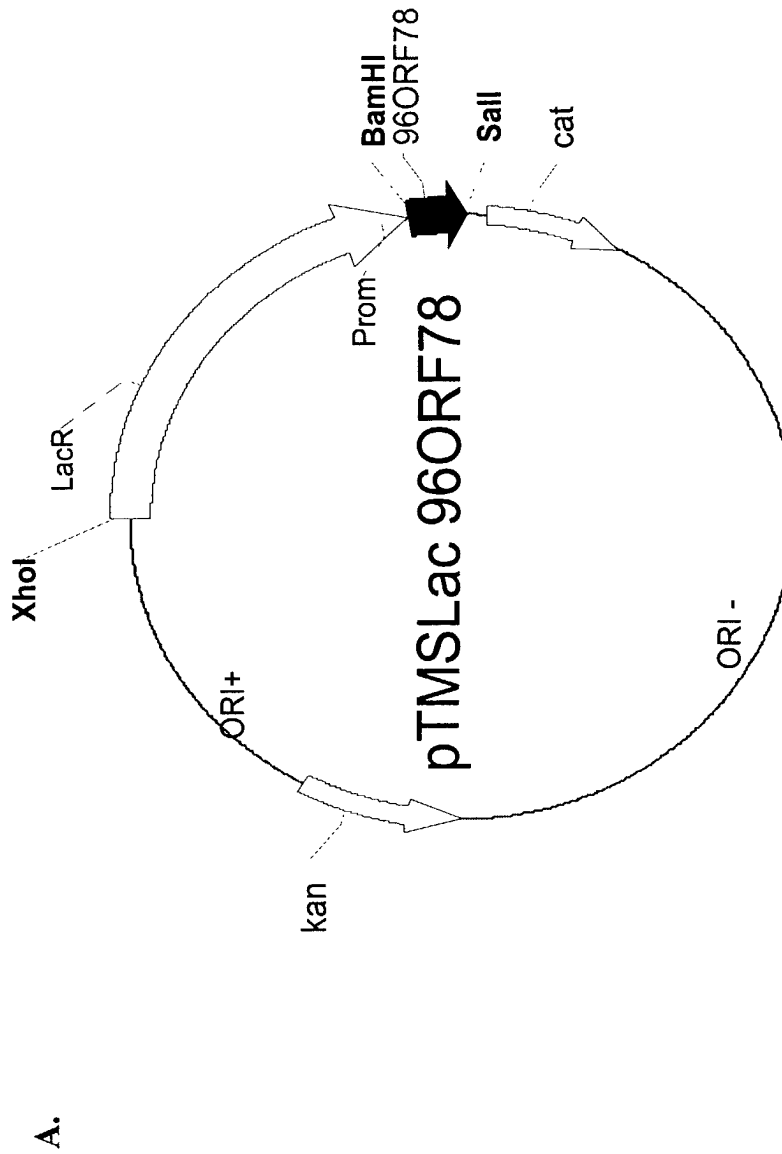
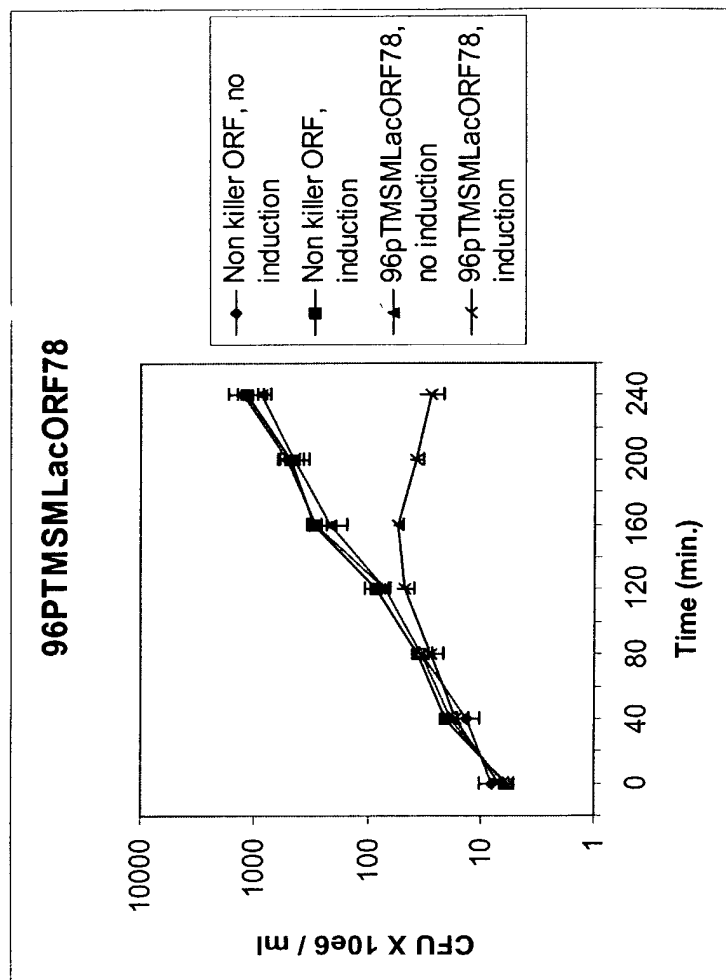


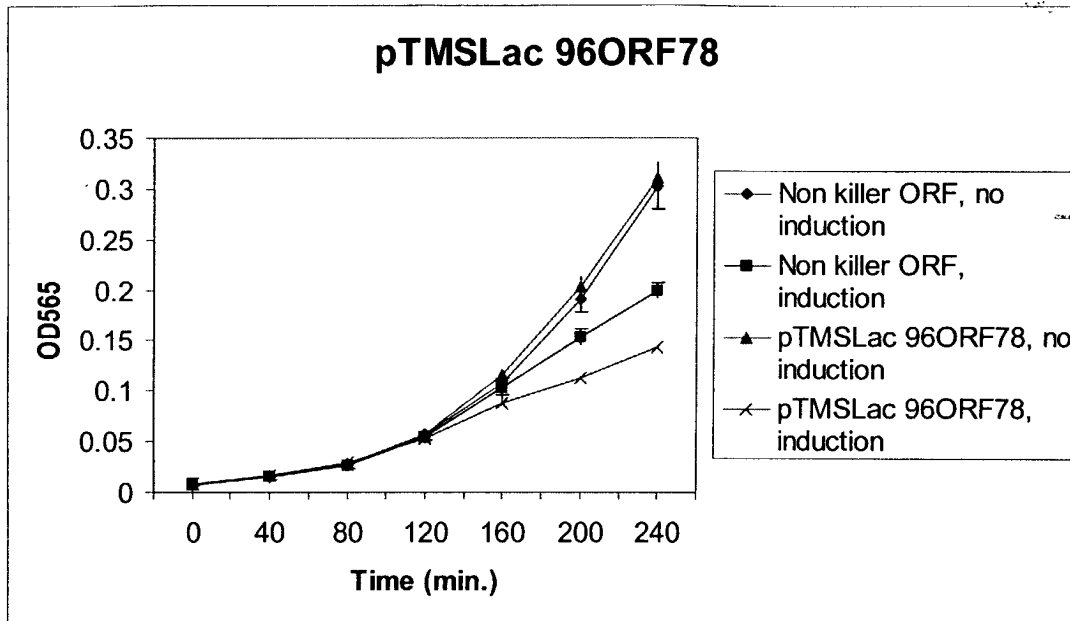
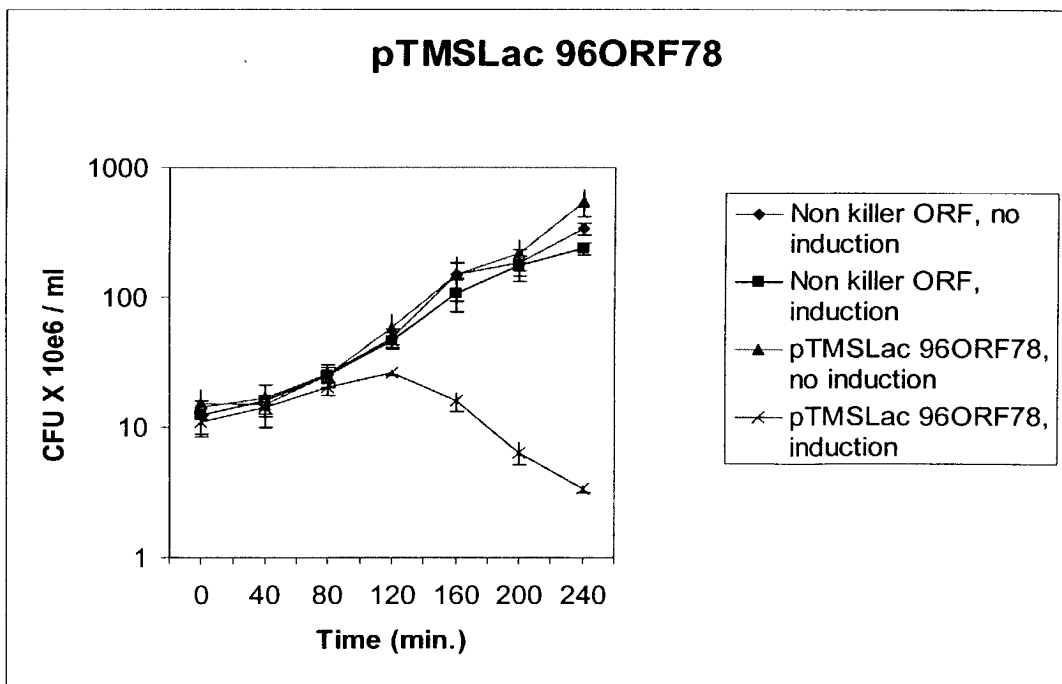
FIGURE 3 B.



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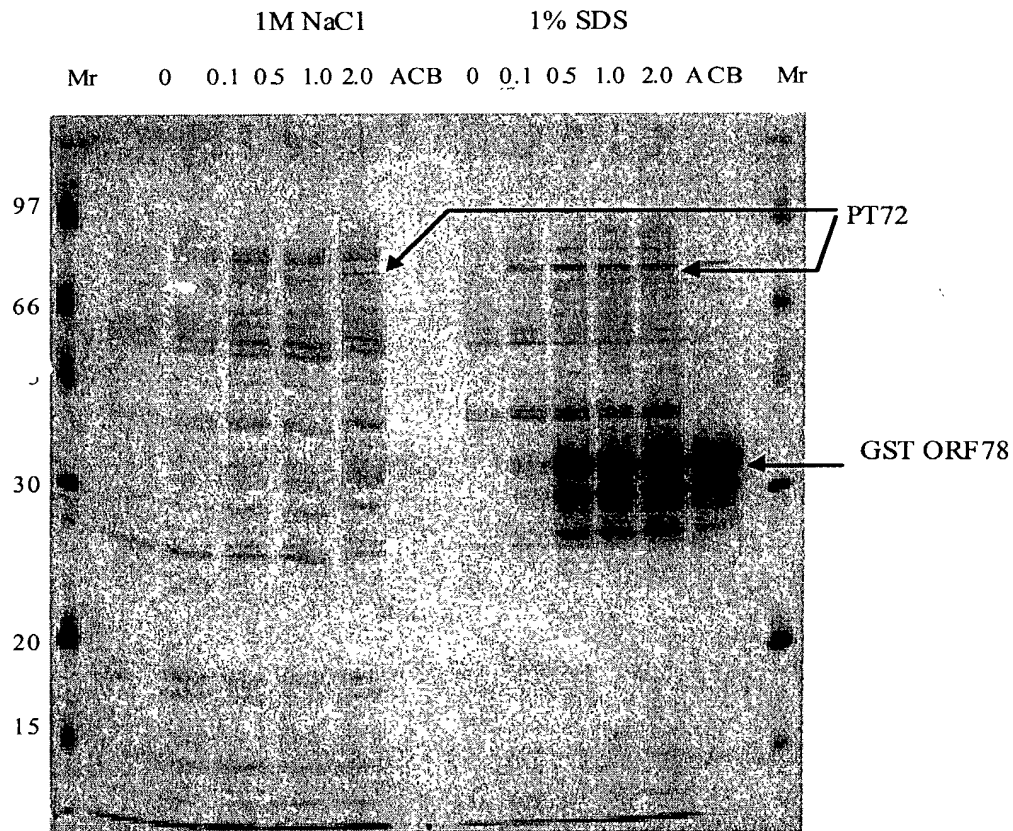
FIGURE 3 C.**D.**

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FIGURE 4

A. GST/96ORF78

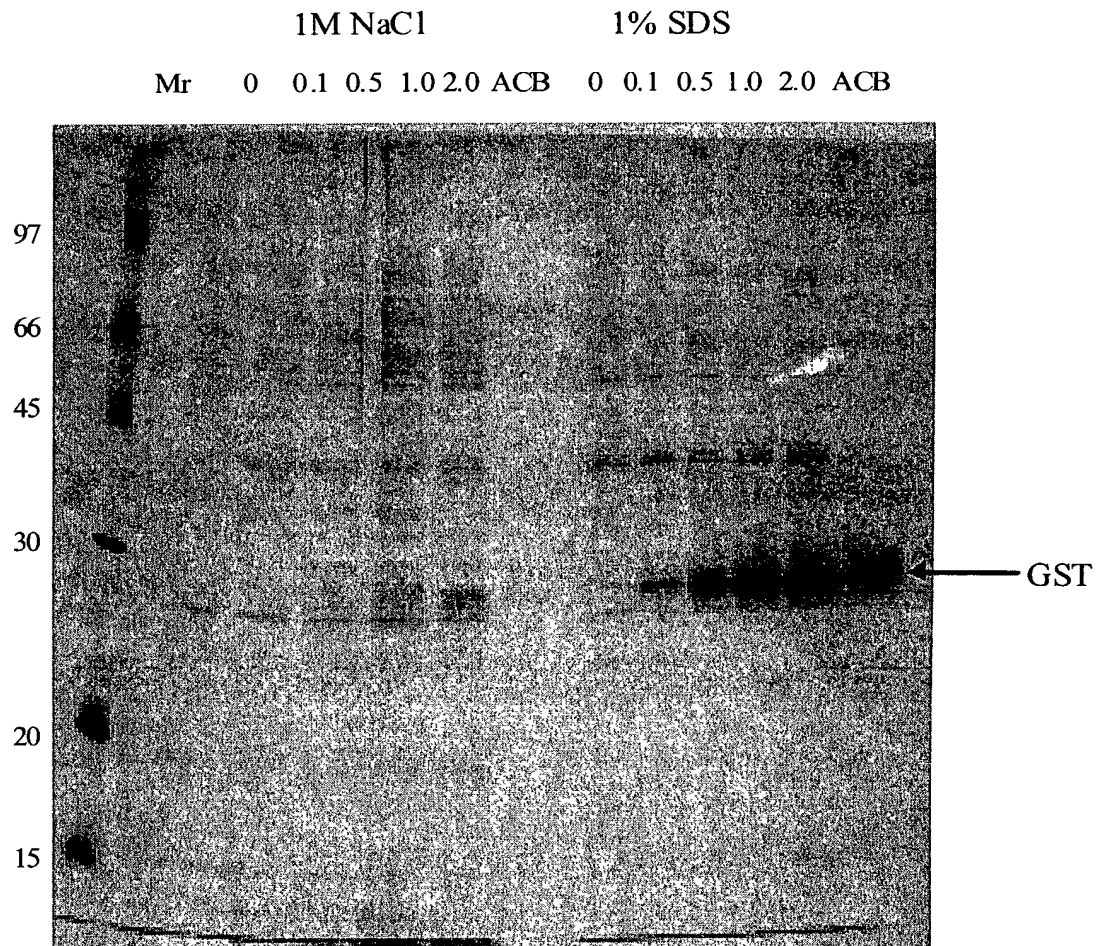


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FIGURE 4

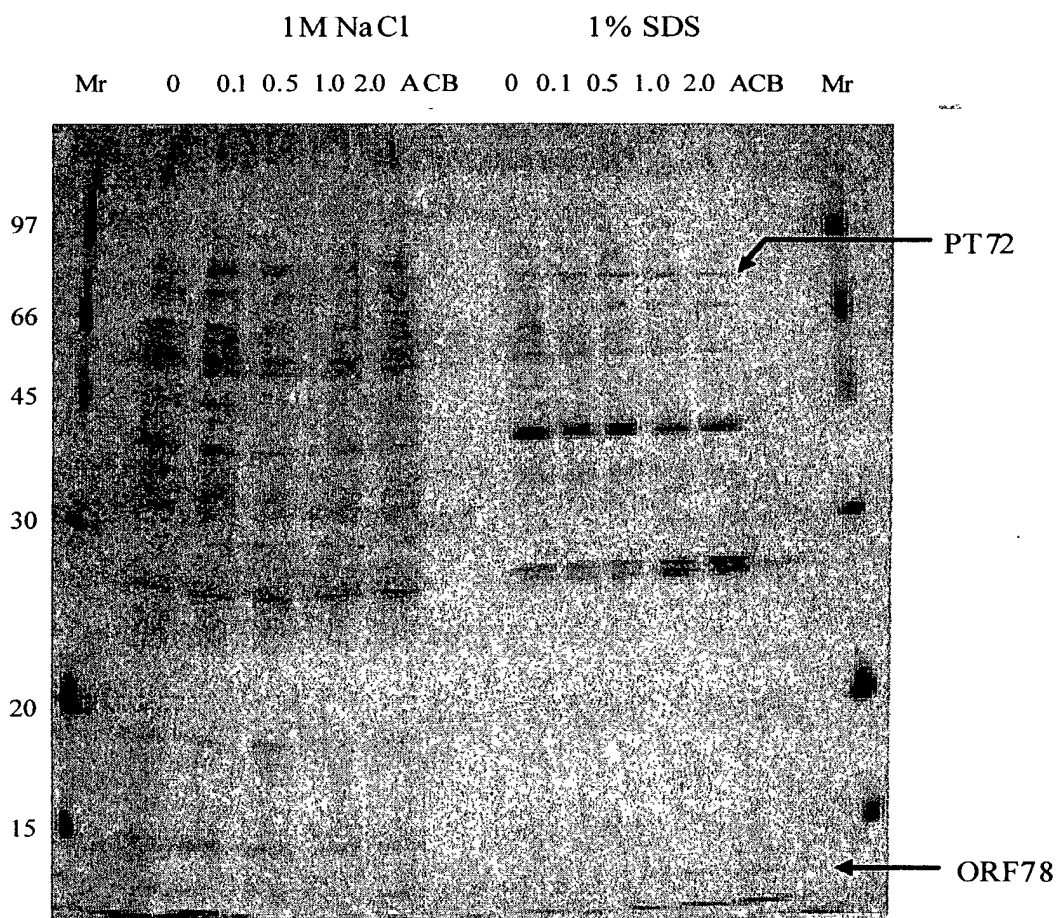
B. GST



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FIGURE 5 96ORF78 (GST removed)

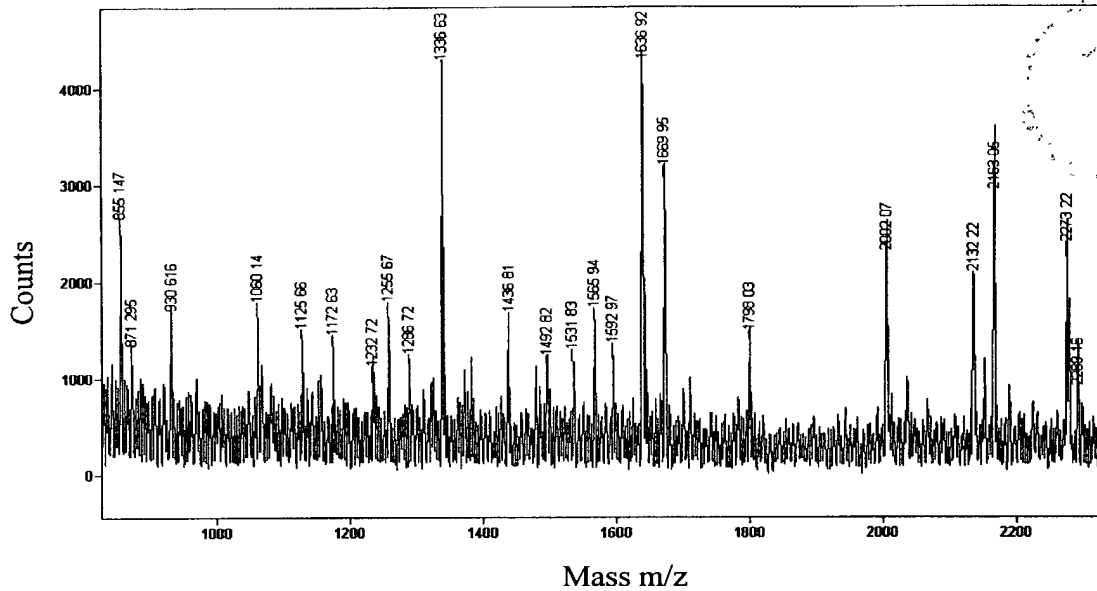


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FIGURE 6



Details for rank 1 candidate in search 20000915095311-0166-127000000001

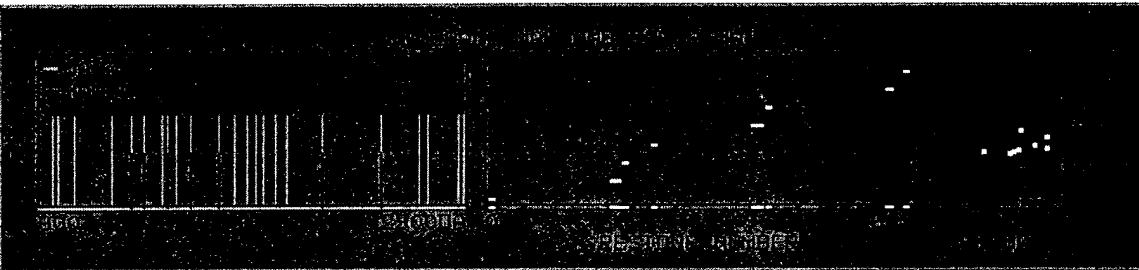
ouContig286: 82020 to 83822: Frame 3: 601 aa

Sample ID: orf78.72 [Pass:0]

Measured peptides: 23

Matched peptides: 7

Min. sequence coverage: 14%



Measured Avg/ Computed Error Residues Missed

Mass(M) Mono Mass (Da) Start To Cut Peptide sequence

1124.649	M	1124.622	0.027	588	596	0	YYLQIVAK
1171.623	M	1171.590	0.033	394	403	0	ELSHDISLMK
1171.623	M	1171.644	-0.021	5	14	0	IDQSHINEIK
1254.661	M	1254.620	0.041	563	573	0	GOETIESLNHK
1335.627	M	1335.536	0.091	193	202	0	NEENFSYYDR
1435.802	M	1435.734	0.068	233	244	0	YLNSPETPIFOK
1797.019	M	1796.930	0.089	177	192	1	KGYDIELAYEAGLLSR
2001.060	M	2001.016	0.044	374	390	1	VSILKDELAHNDLSYER

Unmatched Monoisotopic Masses:

855.147 871.295 930.616 1060.140 1232.717 1286.716 1492.822 1531.830 1565.943 1592.967
1636.921 1669.948 2132.217 2163.048 2273.222 2289.164

FIGURE 7A

A. Pfam HMM search results

Model	Seq-from	Seq-to	HMM-from	HMM-to	Score	E-value
zf-CHC2	3	100	1	98	198.4	1.1e-55
Toprim	260	339	1	151	71.9	1.3e-17

Alignments of top-scoring domains:

zf-CHC2:
domain 1 of 1, from 3 to 100: score 198.4, E = 1.1e-55
*->ipeesIdeLknriDIVdvisYVklKkGrnYkgLCPFHdEKTpSFs
i++++I+e+k+++DI d++seYVkl+K+GrnY+gLCPFHdEKTpSF+
gi|133988 3 IDQSIINEIKDKTDILDVSEYVKLEKGRNYIGLCPFHdEKTpSFT 49

VspeKqfYhCFGCGagGdaIkFlmkyeklsFvEAvekLAdragidlpyek
Vs +Kq+ hCFGc++gG+++ F +++++sFvEAv++L dr+++ ++e
gi|133988 50 VSEDKQICHCFGCKKGGNVFQFTQEIKDISFvEAVKELGDRNVAVDIEA 99

g<-*
+
gi|133988 100 T 100

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Toprim: domain 1 of 1, from 260 to 339: score 71.9, E = 1.3e-17

*->kvliiVEgpsdakalakgkpskrkiyelpgkdgdnvvaslGhlv

+++++ Eg++d+++ a+ +nvva++G+

gi|133988 260 DEIVLLEGFMDVIKSDTAGL-----KNVATMTGTQ- 289

dLptpegyddykwllwlpivdvkkgfepyqiefdqckcskidlkkeql

l++e++

gi|133988 290 -----LSDEHI 295

kllklakkadevilatDpDreGeaiawkllellkpygpveleddkvr

++kl+++ +l++D+D +G ++++k +l+ +q +v++

gi|133988 296 TFIRKLTSN---ITLMFDGDFAGSEATLKTQNLLQQCL-----NVFV 335

iflp<--*

i+lp

gi|133988 336 IQLP 339

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FIGURE 7B

B. Optimal global alignment of amino acid sequences

Sequence 1 sp|O05338|PRIM_STAAU DNA PRIMASE (EC 2.7.7.-) - S. aureus. (572 letters)
Sequence 2 STAAU_R009 STAAU_R009_NT|1-1800| (599 letters)

Identical: 560/605 (92%), Similar: 564/605 (93%), Gap: 39/605 (6%)
seq1 1 M-----IGLCPFHDEKTPSFTVSEDKQICHCF 27
: |||||||
seq2 1 LRIDQSIINEIKDKTDILDVSEYVKLEKGRNYIGLCPFHDEKTPSFTVSEDKQICHCF 60

seq1 28 GCKKGGNVFQFTQEIKDISFVEAVKELGDRNVAVDIEATQSNQVQIASDDDLQMIEMHE 87
|||||
seq2 61 GCKKGGNVFQFTQEIKDISFVEAVKELGDRNVAVDIEATQSNQVQIASDDDLQMIEMHE 120

seq1 88 LIQEFYYVALTKTVEGEQALTYLQERGFTDALIKERGIGFAPDSSHCHDFLQKKGYDIE 147
|||||
seq2 121 LIQEFYYVALTKTVEGEQALTYLQERGFTDALIKERGIGFAPDSSHCHDFLQKKGYDIE 180

seq1 148 LAYEAGLLSRNEENFSYDRFRNRIMFPLKNAQGRIVGYSGRITYTGQEPKYLNSPETPIF 207
|||||
seq2 181 LAYEAGLLSRNEENFSYDRFRNRIMFPLKNAQGRIVGYSGRITYTGQEPKYLNSPETPIF 240

seq1 208 QKRKLLYNLDKARKSIRKLDEIVLLEGFMDVIKSDTAGLKNVVATMGTLSDHEHITFIRK 267
|||||
seq2 241 QKRKLLYNLDKARKSIRKLDEIVLLEGFMDVIKSDTAGLKNVVATMGTLSDHEHITFIRK 300

seq1 268 LTSNITLMFDGDFAGSEATLKTQHLLQQGLNVFVIQLPSGMDPDEYIGKYGNDAFITFV 327
|||||:|||||
seq2 301 LTSNITLMFDGDFAGSEATLKTQGNLLQQGLNVFVIQLPSGMDPDEYIGKYGNDAFITFV 360

1005 5 22 10 02 2001

seq1	328	KNDKKSF	AHKVSI	LKDEIA	NDLSY	ERYLK	ELSHD	ISLMK	SSILQ	QKAI	NDVAP	FFNV	387
											:		
seq2	361	KNDKKSF	AHKVSI	LKDEIA	NDLSY	ERYLK	ELSHD	ISLMK	SSILQ	QKAI	NDVAP	FFNV	420
seq1	388	PEQLANE	IQFNQ	APANY	PEDEY	GGYDE	YGGYI	EP	IGMAQ	FDNLS	RRKEA	ERAF	447
											:		
seq2	421	PEQLANE	IQFNQ	APANY	PEDEY	GGYDE	YGGYI	EP	IGMAQ	FDNLS	RRKEA	ERAF	474
seq1	448	MRDKDT	FLNYY	ESVDK	NFTNQ	HFKYV	FEVLH	DFYA	ENDQ	YNISD	AVQY	VNSNEL	507
seq2	475	MRDKDT	FLNYY	ESVDK	NFTNQ	HFKYV	FEVLH	DFYA	ENDQ	YNISD	AVQY	VNSNEL	534
seq1	508	SLEQYN	LNGEP	YENEI	DDYVN	VINEK	GQETI	ESLN	HKLRE	ATRIG	DVELQ	KYYLQ	567
seq2	535	SLEQYN	LNGEP	YENEI	DDYVN	VINEK	GQETI	ESLN	HKLRE	ATRIG	DVELQ	KYYLQ	594
seq1	568	NKERM											572
seq2	595	NKERM											599

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seq2	358	AGEAGCRRPLVAFKMYLR---RGKNLQHEGERLRYIDEALREIGKLSSPVEQDYLRQL	414
seq1	414	APFFNVSPQLANEIQFNQAPANYYPEDEYGGYIEPEPIGMAQFDNLSRQEKAEAFLLKH	473
seq2	415	AEFSLSLSALHEQLSRQRERTKPREAPDGETARP----MLAKLLLPAFQNAERLILLAH	470
seq1	474	LMRDKDTFLNYYESVDKDNFTNQHFKYVFEVLHDFYAENDQYNISDAVQYVNSNELRETL	533
seq2	471	MMSRDVALVQERIG-GRFNIEHRALAAIYAFYEEGHEADPGALISRI-PGELQPLA	528
seq1	534	ISLEQYNLNDEPYENEIDDYV-NVINEKGQETIESLNHKLREATRIGDVELQKYYLQQIV	592
seq2	529	SDVSLLLIADDVSEQELEDYIRHVLNRPKWLMLKVKEQKTEAERRKDFLTAARIAKEMI	588
seq1	593	AKNK--ERM	599
seq2	589	EMKKMLSSS	597

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[illegible]

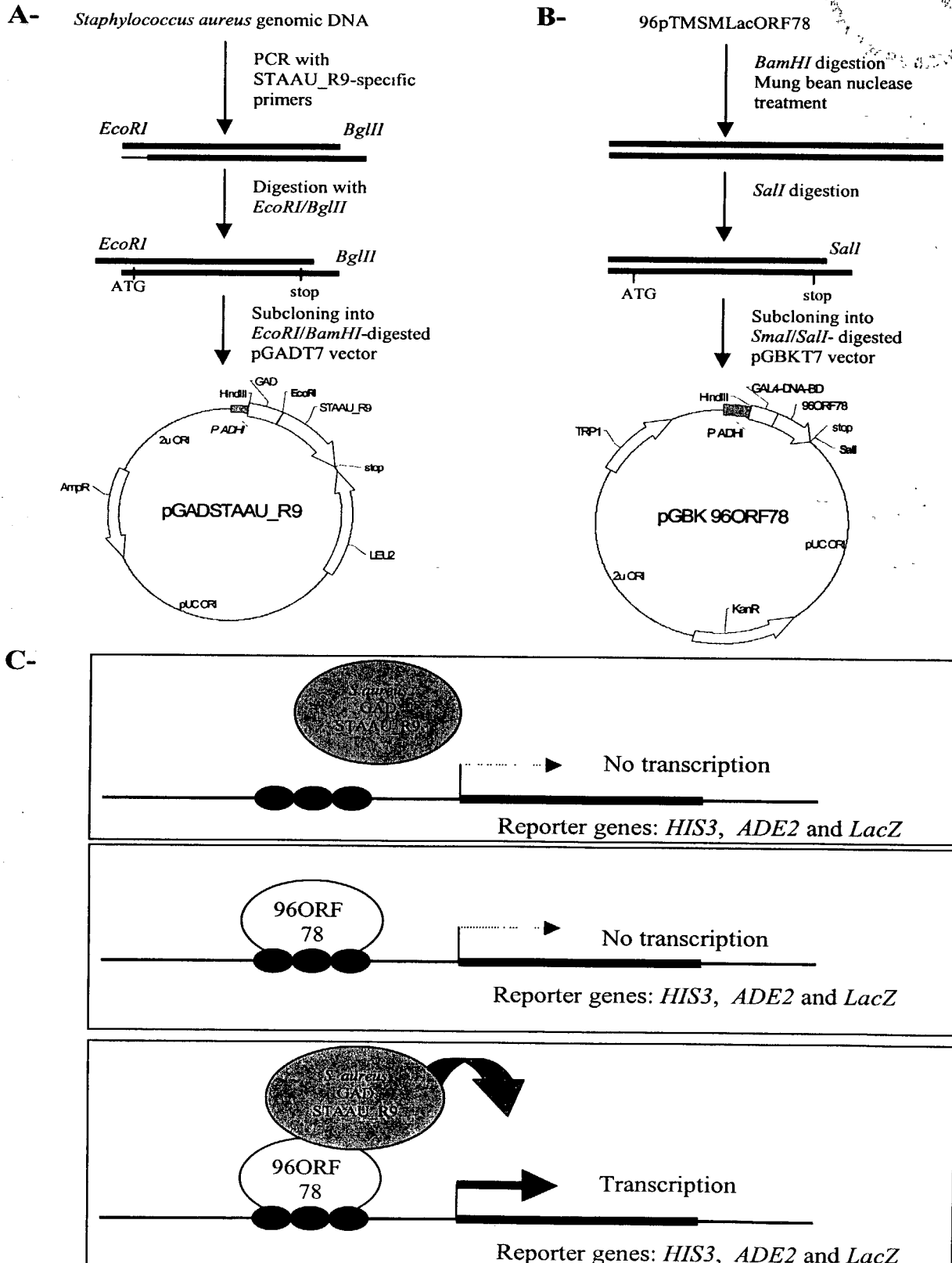
A circular diagram with three points labeled 354, 412, and 406. The points are arranged in a triangle within the circle. Point 354 is at the top left, point 412 is at the bottom center, and point 406 is at the bottom right. The points are connected by lines forming a triangle.

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seq1	413	VAPFFNVSPEQLANEIQF--NQAPANNYPPEDEYGGYIEPEPIGMAQFDNLSRQEKAERAF	470
	:	: : : : : :	:
seq2	407	LRIYLR---QELGNKLGILDDSQLERLMPKAAESGVS RVP-----QLKRRTMRIL	454
	:	: : : : : :	:
seq1	471	LKHLMRDKD--TFLNYYESVDKDNFTNQHFKYVFEVLHDFYAENDQYNISDAVQYVNSNE	528
	:	: : : : : :	:
seq2	455	IGLLVQNPELATLVPPLNLDENKLPGLG---LFREL VNTCLS QPGLTTGQLLEHYRGTN	511
	:	: : : : : :	:
seq1	529	LRETLISLEQY-----NLNDEPYENEIDDYVNVINEKGQETIESLNHKLREATR--IGD	580
	:	: : : : : :	:
seq2	512	NAATLEKLSMWDDIADKNIAEQFTDLSLNHMFDSLLELRQEELIA-----RERTHGLSNE	566
	:	: : : : : :	:
seq1	581	VELQKYYLQQIVAKNKERM	599
	:	: : : : : :	:
seq2	567	ERLELWTLNQELAKK----	581
	:	: : : : : :	:

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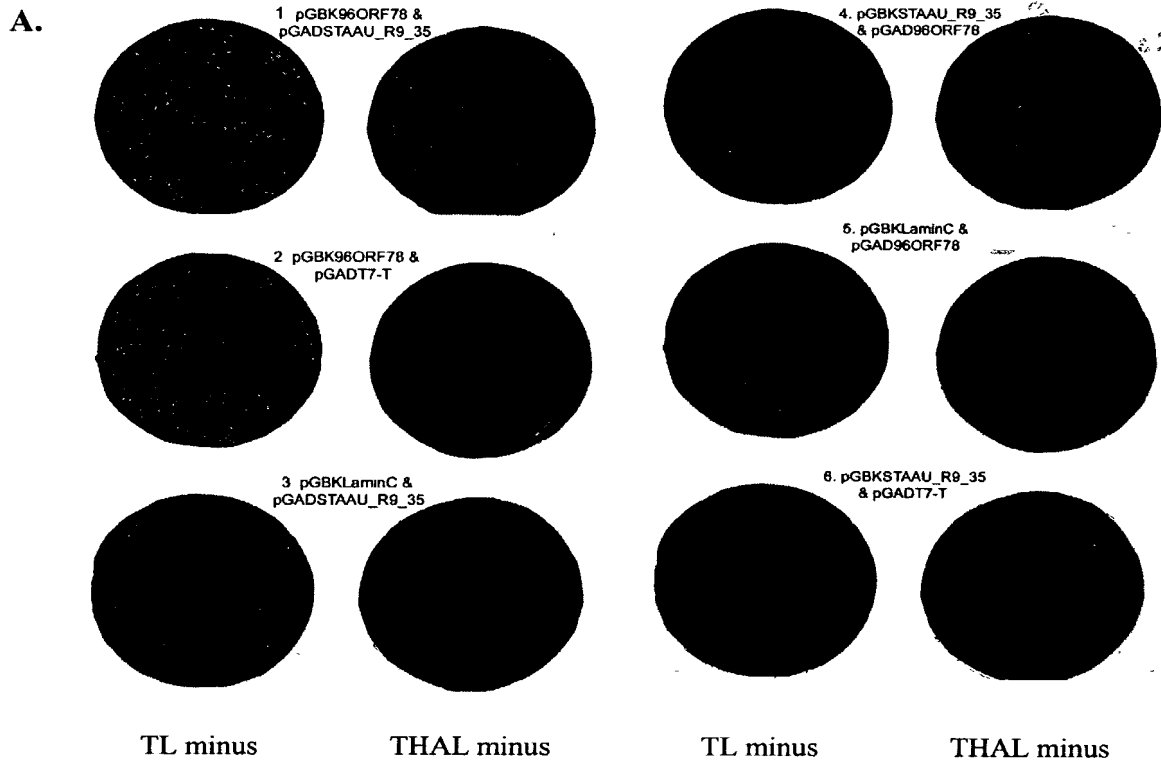
FIGURE 8



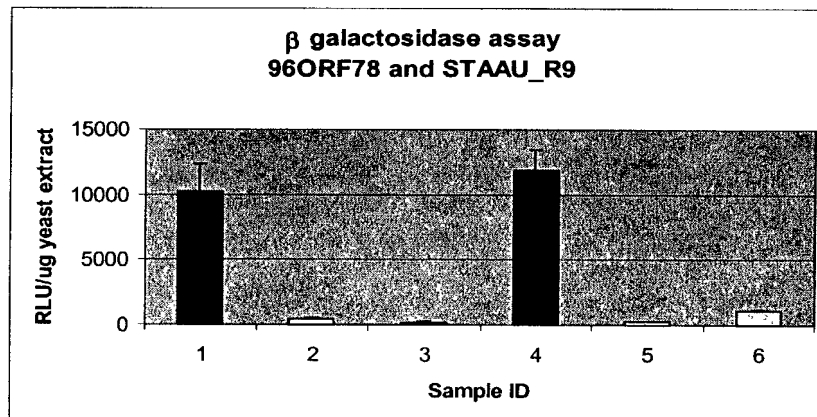
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FIGURE 9



B.



Sample	Yeast co-transformants		RLU (avg)	SD
	Plasmid 1	Plasmid 2		
1	pGBK 96ORF78	pGADSTAAU_R9_35	10,250	2,080
2	pGBK 96ORF78	pGADT7-T	475	38
3	pGBK Lamin C	pGADSTAAU_R9_35	224	10
4	pGBKSTAAU_R9_35	pGAD 96ORF78	11,935	1,477
5	pGBK Lamin C	pGAD 96ORF78	243	12
6	pGBKSTAAU_R9_35	pGADT7-T	1,121	37

FIGURE 10

A. Fragments of STAAU_R9

B.

Interaction with
96ORF78

599

1

Yes

35

599

35

Yes

35

342

No

229

402

No

229

599

Yes

380

599

Yes

380

449

No

380

490

No

380

530

No

380

561

No

449

599

Yes

490

599

Yes

530

599

Yes

561

599

Yes

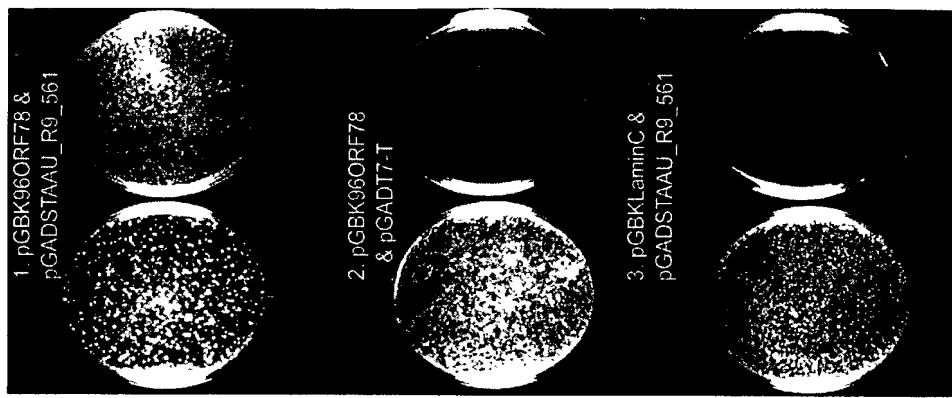
SEQ ID NO: 6 STAAU_R9_561-599
AMINO ACID SEQUENCE

QGETIESLNHLKREATRIGDVELQKYLLQIVAKNKERM

SEQ ID NO: 5 STAAU_R9_1683-1800
NUCLEIC ACID SEQUENCE

GGACAAGAAACAATTGAGTCATTGAATCATATAAATTAAAGGGAAGCTACAAGGATTGGCGATGTA
GAATTACAAAATACTATTACAGCAAATTGTTGCTAAGAATAAAGAACCGCATGTAG

C.



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FIGURE 11

A.

	Primer name	Primer sequence	Restriction site
SEQ ID NO: 8	R9_5E3	5'-ccggaattcTTGCGAATAGATCAATCG-3'	EcoRI
SEQ ID NO: 9	R9_3BG	5'-ggaagatctCTACATGCGTTCTTTATTC-3'	BglII
SEQ ID NO: 10	R9_5E	5'-ccggaattcATGATAGGTTTGTGTCCT-3'	EcoRI
SEQ ID NO: 11	R9_5E1	5'-ccggaattcCCAAAATACCTAAATAGTCC-3'	EcoRI
SEQ ID NO: 12	R9_5E2	5'-ccggaattcGCACATAATGACCTTTCA-3'	EcoRI
SEQ ID NO: 13	R9_342R	5'-cgcgatccATGCCTGATGGCAATTG-3'	BamHI
SEQ ID NO: 14	R9_402R	5'-ccatcgatGATTCATAAGCGAAATATC-3'	Clal
SEQ ID NO: 15	R9_449F	5'-ccggaattcCCTGAGCCAATTGGTATGGC-3'	EcoRI
SEQ ID NO: 16	R9_449R	5'-cgcgatccctaAGGTTCAATGTAACCGCC-3'	BamHI
SEQ ID NO: 17	R9_490F	5'-ccggaattcAAGGATAACTTCACAAATCAG-3'	EcoRI
SEQ ID NO: 18	R9_490R	5'-cgcgatccctaCTTATCAACACTTTCATAATA-3'	BamHI
SEQ ID NO: 19	R9_530F	5'-ccggaattcAGAGAAACACTAATTAGCTTA-3'	EcoRI
SEQ ID NO: 20	R9_530R	5'-cgcgatccctaTCTCAACTCATTTGAATTAAC-3'	BamHI
SEQ ID NO: 21	R9_561F	5'-ccggaattcGGACAAGAAACAATTGAGTC-3'	EcoRI
SEQ ID NO: 7	R9_561R	5'-cgcgatccctaTCCTTTTTCATTAATAACATTG-3'	BamHI

B.

Cloning of SEQ ID NO: 2 amino acid fragments	Sense primer	Antisense primer
1-599	R9_5E3	R9_3BG
35-599	R9_5E	R9_3BG
35-342	R9_5E	R9_342R
229-402	R9_5E1	R9_402R
229-599	R9_5E1	R9_3BG
380-599	R9_5E2	R9_3BG
380-449	R9_5E2	R9_449R
380-490	R9_5E2	R9_490R
380-530	R9_5E2	R9_530R
380-561	R9_5E2	R9_561R
449-599	R9_449F	R9_3BG
490-599	R9_490F	R9_3BG
530-599	R9_530F	R9_3BG
561-599	R9_561F	R9_3BG